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Anemia among pregnant women attending primary healthcare units in the municipality of São Paulo, Brazil: evaluations after the mandatory fortification of wheat and maize flours with iron

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Abstract

Background: Improvements in the iron status of a population as result of food fortification are expected at the long term. In Brazil, the effectiveness of mandatory flour fortification with iron has been evidenced mostly from surveys on gestational anemia after 1 or 2 years from its implementation, in 2004. Our aim was to assess hemoglobin (Hb) concentrations and the prevalences of anemia and linked erythrocyte morphology patterns among pregnant women in 2006 and 2008.

Methods: The study design was retrospective and cross-sectional. The analysis was based on secondary data in 546 medical records from women at the times of their first prenatal attendance in 13 public primary healthcare units of the Butantan Administrative Region from São Paulo (SP), Brazil. Anemia was evaluated from Hb concentration (cut-off <11.0 g/dL) and erythrocyte morphology patterns from mean corpuscular volume (MCV), mean corpuscular Hb concentration (MHC), and red cell distribution widths (RDW). Recorded sociodemographic and obstetric data included maternal age, gestational age, ethnoracial self-classification, and residence type. Student's *t* tests, analysis of variance, Chi-squared tests, and multiple linear and logistic regressions were employed in the statistical analysis using a significance level of 5 %.

Results: The prevalence of anemia was 9.7 % in 2006 and 9.4 % in 2008 ($P = 0.922$), with no significant difference in mean Hb concentrations ($P = 0.159$). Normocytosis (normal MVC), normochromia (normal MHC), and anisocytosis (high RDW) were found in most anemia cases, suggesting that the low Hb concentrations resulted from mixed causes. In multiple regression analysis, gestational age at the first prenatal attendance was an independent predictor of low Hb and of having anemia. Moreover, black ethnoracial self-classification was associated with lower Hb.

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Conclusions: The prevalence of gestational anemia was low among women in both of the studied periods, representing a mild public health problem. Our results highlight the importance of early prenatal care as a means of reducing gestation-associated risks. The erythrocyte morphology pattern found in most cases of low Hb levels suggests that, besides iron deficiency, hemoglobinopathies and nutritional deficiencies of folate and vitamin B12 are common complicating factors of gestational anemia in our setting.

Keywords: Hemoglobin, Erythrocyte morphology parameters, Iron deficiency anemia, Pregnancy, Prenatal care

Background

Women with low body iron stores at the beginning of pregnancy are at a considerable risk of developing iron deficiency anemia because the body's requirement for this mineral substantially increases with gestation [1]. Of the total amount of iron that is needed by an adult mother and her fetus during normal pregnancy, more than 90 % must be available during the second and third trimesters, which corresponds to about 5.5 mg of iron daily [2]. Because the bioavailable iron from women's diets and their pre-gestational body iron stores are typically insufficient to meet this high requirement, it is of paramount importance to ensure adequate iron supplementation for pregnant women [3].

Based on these facts, the Brazilian Ministry of Health has been encouraging iron supplementation during pregnancy for at least the last three decades [4]. In 2005, this initiative was reinforced by the National Iron Supplementation Program (*Programa Nacional de Suplementação de Ferro*), which aimed to ensure prophylactic iron supplementation for all pregnant women after 20 weeks of gestation [5]. The diagnosis of anemia in this group has also been a top priority for public health programs, as evidenced by its inclusion as a preliminary obligation for healthcare units in the context of the Humanization of Prenatal and Childbirth Care Program (*Programa de Humanização do Pré-Natal e Nascimento*) [6].

As a complementary action in the Brazilian government's efforts to fight iron deficiency, iron fortification of wheat and maize flours has been mandated in all national territories since July 2004 [7]. According to this program, a daily intake of 100 g of flour should provide at least 30 % of the recommended dietary allowance (RDA) of iron for a healthy adult [8], which is expected to reduce the prevalence of nutritional iron inadequacy in the population. However, following the program's implementation, there has been no internal evaluation of body iron status in the target population groups. Furthermore, there have been contradictory findings regarding the changes in anemia prevalence during the post-fortification period, which have prevented definitive conclusions from being reached on this issue [9–14].

In a nationwide evaluation of women at their first prenatal attendance in public healthcare facilities, Fujimori et al. reported a fall in the prevalence of anemia after flour fortification, especially in the northeast and north regions of Brazil. During the pre-fortification period, the frequency of anemia cases varied from 32.2 to 37.4 % in these areas [11]. Similar findings were reported for pregnant women from Rio de Janeiro (RJ) in the southeast region [12]. On the other hand, the anemia prevalence rates in the same groups of women from the cities of São Paulo (SP) and Maringa (PR) did not seem to differ between the post-fortification period (8.6 and 9.4 %) and the pre-fortification period (before 2004, 9.2 and 12.3 %) [10, 13].

Although iron deficiency is considered to be the cause of 50 % of anemia cases in the world, this proportion varies markedly across different populations [15, 16]. Furthermore, it is described that for a given prevalence of iron deficiency anemia, about two to five times more individuals are at risk for iron deficiency without anemia [17]. Hence, a reliable assessment of body iron status depends not only on Hb but also on other more expensive laboratory assays such as serum ferritin, serum transferrin receptor, and transferrin saturation index [18]. The parameters of erythrocyte morphology, such as mean cell volume (MCV), mean cell Hb (MCH), and red cell distribution width (RDW), provide cheaper markers of late changes in iron status. Usually, severe cases of iron deficiency present microcytosis (low MCV), hypochromia (low HCM), and anisocytosis (high RDW) [19]. Monitoring the hemoglobin (Hb) response to an increase in the iron intake from dietary sources or supplementation can further proof the diagnosis of iron deficiency anemia [17].

The aim of the present study was to assess Hb concentrations and the prevalence of anemia as well as their associated factors among pregnant women evaluated at the times of their first prenatal attendance in primary healthcare units from the Butantan Administrative Region (São Paulo municipality, Brazil) during the years 2006 and 2008. We have also described the erythrocyte morphology patterns that were associated with anemia during these two periods, which corresponded to the second and fourth years after the effective implementation of mandatory iron fortification of flours in Brazil.

Methods

Study population

The study design was retrospective and cross-sectional. The analysis relied on secondary data that had been obtained from the medical records of pregnant women who were attending first prenatal care appointments at 13 primary healthcare units in the Butantan Administrative Region (São Paulo) during 2006 and 2008. This Region, which covers an area of 56.1 km^2, belongs to one of five districts that are controlled by the municipality's Centre-West Regional Health Care Coordination center. Each primary healthcare unit attends to the needs of the population according to guidelines from the Brazilian Ministry of Health concerning public health and safety, disease prevention, diagnosis, treatment, and rehabilitation. In 2008, the primary healthcare units from the Butantan Administrative Region provided prenatal care services for around 3700 pregnant women.

To calculate the study sample size, we used the formula $n = [z^2 \times p \times (1 - p)] \times d^{-2}$ [20], where p is the expected estimate (0.086), z is the percentile of the normal distribution (1.96), and d is the maximum absolute error (0.035). Based on this formula, a minimum sample of 247 individuals was established for each year of the study. The expected prevalence was obtained in a previous study that had been conducted with part of the target population between 2005 and 2006 [10].

Because improvements in iron status were expected to result from long-term increases in iron intake from fortified foods [21], a 2-year interval was chosen for the assessment. Therefore, pregnant women who were attended by the primary healthcare units in 2007 were not included in the study.

Data collection

In order to avoid sample losses, 500 medical records of pregnant women from each year of the study were initially sorted by random draw from the Butantan Administrative Region's central databank. In a first selection step, medical records concerning first prenatal care assistance entered in the study's databank only if they described the following: (1) maternal age; (2) date of last menstruation; (3) complete laboratorial results for Hb, MVC, MCH, and RDW; (4) absence of diabetes, hypertension, or liver or kidney pathology diagnosis; and (5) no history of use of iron-containing supplements. Then, of the resulting medical records ($n = 772$), just those that also showed complete information about the woman's ethnoracial self-classification and residential building type were selected for the final sample. With the data that were retained in the first selection step, comparisons of obstetric and hematologic variables between women included or not the final sample were carried out in order to check any sampling bias possibly driven by exclusion of records with inconsistent sociodemographic information.

The number of days between the last menstruation and the blood collection was used to estimate the gestational age at the time of the first prenatal care attendance and to assign the corresponding trimester of pregnancy as first (<13 weeks), second (13–27 weeks), or third (>27 weeks) [22]. Women younger than 20 years old were considered adolescent mothers. Ethnoracial self-classifications were based on the categories adopted by the Brazilian Institute of Geography and Statistics (IBGE) in census surveys: white, mixed ("pardo" in Portuguese), black, yellow (Asian Brazilian), or indigenous (Native American) [23]. The type of current residence was categorized as a brick house/apartment or as other type of housing, according to reported characteristics from the domicile (house or apartment, slum or tenements) and the building materials that had been used to build the walls (brick or wood). When available in the medical records, data regarding maternal schooling (<8 or ≥ 8 years of study), occupation (remunerated or non-remunerated job), and marital status (with or without a partner) were also obtained.

Hematological determinations were made by the Butantan Administrative Region's central laboratory with the aid of an automatized and daily-calibrated Sysmex XE-2100D system (Roche Diagnostics, Meylan, France), based on blood samples from individuals who had fasted for at least 8 h. The occurrence of anemia was evaluated from Hb concentration using the pregnant specific cut-off (<11.0 g/dL) recommended by the World Health Organization (WHO) and the Brazilian Ministry of Health [24, 25]. Age-specific WHO reference values were also used to define normality for MCV (78–98 and 81–99 fL for 15- to 17.9- and ≥18-year-old women, respectively) and MCH (26–34 pg, irrespectively of age) [24]. Anisocytosis was considered present for RDW >14 % [25].

Statistical analysis

Data analysis was performed with the Statistical Package for Social Sciences (SPSS), version 20.0 (IBM Corp., Armonk, USA). The normality and homoscedasticity of the distributions of continuous variables were assessed using Kolmogorov-Smirnov and Levene tests, respectively. Descriptive data were summarized as means and standard deviations (SDs) or as absolute and relative frequencies. Student's t tests, analysis of variance (ANOVA) followed by Tukey's post hoc tests, and Chi-squared tests were used to detect bivariate associations ($P < 0.05$, two-sided).

To assess possible predictors of Hb concentration and anemia prevalence, multiple linear and logistic regression analysis were employed using the stepwise method. Initially, the following variables were used in the regression

model: year group (2008 vs. 2006), maternal age (years), gestational age (weeks), ethnoracial self-classification (black vs. white and mixed), and residence type (brick house/apartment vs. other types of housing). Subsequently, variables with non-significant associations ($P > 0.05$) were sequentially removed. There was no substantial change in model parameters after the removal or inclusion of non-significant variables, and we decided to present β values, odds ratios (OR), and corresponding 95 % confidence intervals for the original models (those with all variables included).

Goodness-of-fit was checked for the multiple logistic and linear regression models by using Hosmer and Lemeshow's test and by analyzing the residuals (assessing normality and checking for visual patterns in the distribution of plotted values), respectively.

Results

Of 1000 medical records initially sorted, 772 were retained in the first sampling step, and 546 in the second one. These were included in the final sample, which comprised 259 records from 2006 and 287 records from 2008. No significant difference of maternal ages, gestational ages, and hematological parameters were observed between women included in the final sample and those excluded due to incomplete sociodemographic data ($P > 0.05$, data not shown). Homogenous distributions of the gestational period, maternal age, ethnoracial self-classification, and residence type categories were also observed in the two studied year groups (Table 1). The mean maternal and gestational ages at first prenatal attendance in 2006 and 2008 were 25.3 ± 6.0 and 26.1 ± 6.5 years ($P = 0.123$) and 15.5 ± 7.3 and 16.2 ± 7.6 weeks ($P = 0.260$), respectively.

More than half of the women self-reported their ethnoracial group as white, and more than two thirds resided in a brick house or apartment. No woman self-reported her ethnoracial group as yellow or indigenous. Although some of the medical records were incomplete regarding maternal schooling, occupation, and marital status, the available data indicated that the proportion of women who had completed elementary schooling (≥8 years of study) was lower in the 2006 group than in the 2008 group. Nonetheless, in the latter group, a significantly higher proportion of women had a non-remunerated job. Approximately half of the women reported that they did not have a current partner (Table 1).

In both the 2006 and 2008 groups, average Hb concentrations varied across the gestational period categories; however, no difference was found when the values from the 2 years were compared. Accordingly, the overall prevalences of anemia did not differ between 2006 and 2008 groups (9.7 vs. 9.4 %, $P = 0.922$). Women whose first prenatal attendance occurred during the third trimester of pregnancy had the highest anemia prevalences (40.0 % in 2006 and 23.5 % in 2008) (Table 2).

In all gestational periods, about one third of the anemic women showed a microcytic-hypochromic erythrocyte morphology pattern. No macrocytic (high MCV) case of anemia was found. Anemia and anisocytosis accounted for 46 and 63 % of all normocytic-normochromic and microcytic-hypochromic cases and had a prevalence of 3.8 % in the 2006 group and 5.2 % in the 2008 group

Table 1 Descriptive characteristics of pregnant women at first prenatal attendance in 2006 and 2008

Demographic characteristics[1]	2006		2008		*P* value[2]
	%	*N*	%	*n*	
Maternal age					
< 20 years	16.6	43	17.8	51	0.718
≥ 20 years	83.4	216	82.2	236	
Total n		259		287	
Gestational period					
First trimester	47.1	122	40.4	116	0.140
Second trimester	45.2	117	47.7	137	
Third trimester	7.7	20	11.8	34	
Total n		259		287	
Ethnoracial self-classification					
White	54.4	141	52.3	150	0.549
Mixed ("Pardo")	39.0	101	38.7	111	
Black	6.6	17	9.1	26	
Total n		259		287	
Residence type					
Brick house/apartment	74.9	194	68.6	197	0.105
Another type of housing	25.1	65	31.4	90	
Total n		259		287	
Schooling					
< 8 years of study	59.0	131	37.6	99	*<0.001*
≥ 8 years of study	41.0	91	62.4	164	
Total n		222		263	
Occupation					
Remunerated job	84.1	175	57.1	117	*<0.001*
Non-remunerated job	15.9	33	42.9	88	
Total n		208		205	
Marital status					
With a partner	55.5	116	48.6	101	0.156
Without a partner	44.5	93	51.4	107	
Total n		209		208	

[1]Information on schooling, occupation, and marital status were not available in all of the studied medical records

[2]*P* values refer to comparisons of category frequencies between the groups, using Chi-squared tests. *P*-value in italics indicates statistical significances

Table 2 Hemoglobin (g/dL) and prevalence of anemia among women at first prenatal attendance by gestational trimester in 2006 and 2008

Variable	2006		2008		*P* value[1]
	Mean	SD	Mean	SD	
Hemoglobin					
First trimester	12.65[a]	1.00	12.58[a]	1.03	0.597
Second trimester	12.16[b]	1.08	12.04[b]	0.91	0.252
Third trimester	11.40[c]	1.26	11.58[c]	1.04	0.572
Total	12.33	1.11	12.20	1.03	0.159
	2006		2008		*P* value[2]
	%	*n*	%	*n*	
Anemia					
First trimester	4.9[a]	6	5.2[a]	6	0.929
Second trimester	9.4[a]	11	9.5[a]	13	0.981
Third trimester	40.0[b]	8	23.5[b]	8	0.201
Total	9.7	25	9.4	27	0.922

[1]*P* values refer to comparisons of means between groups, using ANOVA tests
[2]*P* values refer to comparisons of category frequencies between groups, using Chi-squared tests
[a,b,c]Means or category frequencies with different superscripts within the same column are significantly different according to Tukey's *post hoc* or Chi-squared tests
SD standard deviation

($P = 0.439$). The prevalence of microcytic-hypochromic anemia did not differ significantly between the 2-year groups (3.9 % in 2006 vs. 2.1 % in 2008, $P = 0.221$). On the other hand, in the descriptive analysis, the frequency of microcytic-hypochromic anemia was found to be about three times higher among women who self-reported as black, when compared those who self-reported as white or mixed (Table 3).

Multiple regression analysis corroborated the influence of gestational age on Hb concentrations and presence of anemia. A fall of 0.04 g/dL in Hb concentration and an increase of 8.9 % in the probability of having anemia were observed for each additional week of gestational age that had elapsed before the beginning of prenatal care. Moreover, independently of other tested variables, women who self-reported as black showed Hb concentration 0.34 g/dL lower than that estimated for women who self-reported other ethnoracial categories (white and mixed) (Table 4).

Discussion

By surveilling trends in the diet and health status of target population groups, valuable information can be obtained on the expected impact of large-scale nutrition interventions [26]. Here, we assessed anemia prevalence among pregnant women who attended primary healthcare units in the region of Butantan (São Paulo-SP, Brazil) after 2 and 4 years after the implementation of the national flour iron fortification program. We observed that anemia prevalence did not differ among women from 2006 and from 2008 and that it was a mild public health problem in both periods (overall prevalences <10 %) according to the WHO criteria for the classification of epidemiological severity [17].

Our results are similar to those by Sato et al. in a comparison of pre- and post-fortification prevalence rates of

Table 3 Hemoglobin (g/dL) and prevalence of anemia among women at first prenatal attendance by descriptive characteristics

Demographic characteristics	*n*	Hemoglobin			Anemia			Microcytic-hypochromic anemia		
		Mean	SD	*P* value[1]	%	*n*	*P* value[2]	%	*n*	*P* value[2]
Maternal age										
Adolescents	94	12.21	1.04	0.578	8.5	8	0.713	3.2	3	0.870
Adults	452	12.27	1.08		9.7	44		2.9	13	
Gestational period										
First trimester	238	12.61[a]	1.02	*<0.001*	5.0[a]	12	*<0.001*	1.7[a]	4	*0.011*
Second trimester	254	12.10[b]	0.99		9.4[a]	24		2.8[a]	7	
Third trimester	54	11.52[c]	1.12		29.6[b]	16		9.3[b]	5	
Ethnoracial self-classification										
White	291	12.29	1.03	0.150	8.2	24	0.426	2.1[a]	6	*0.031*
Mixed ("Pardo")	212	12.30	1.14		10.4	22		2.8[a]	6	
Black	43	11.96	1.02		14.0	6		9.3[b]	4	
Residence type										
Brick house/apartment	391	12.23	1.03	0.262	9.0	35	0.469	3.1	12	0.757
Another type of housing	155	12.35	1.17		11.0	17		2.6	4	

[1]*P* values refer to comparisons of means between groups, using Student's t or ANOVA tests. P-values in italics indicate statistical significance
[2]*P* values refer to comparisons of frequencies between groups, using Chi-squared tests. *P*-values in italics indicate statistical significance
[a,b,c]Means or category frequencies with different superscripts within the same column are significantly different according to Tukey's *post hoc* or Chi-squared tests
SD standard deviation

Table 4 Multiple linear and logistic regression models of predictors of Hb concentrations and anemia prevalence

Variable	Hemoglobin model[1]			Anemia model[2]		
	β	95 % CI	*P* value	OR	95 % CI	*P* value
Year group (2008 *vs.* 2006)	-0.102	-0.275; 0,070	0.244	0.870	0.481; 1.564	0.646
Maternal age (years)	0.006	-0.008; 0.019	0.429	1.015	0.970; 1.062	0.510
Gestational age (weeks)	-0.044	-0.055; -0.032	*<0.001*	1.089	1.053; 1.127	*<0.001*
Ethnoracial self-classification (black *vs.* white and mixed)	-0.337	-0.656; -0.018	*0.039*	1.858	0.722; 4.785	0.199
Residence type (another type of housing *v.* brick house/apartment)	0.133	-0.058; 0.323	0.171	1.282	0.681; 2.4115	0.441

[1]Multiple linear regression with Hb concentration (g/dL) as the dependent variable (n = 546).P- value in italics indicates statistical significance
[2]Multiple logistic regression with anemia (Hb < 11 g/dL) as the dependent variable (n = 546). Hosmer & Lemeshow: $P = 0.176$
OR odds ratio, *CI* confidence interval

anemia among pregnant women attending a primary healthcare unit from the same region [10]; and also to those by Guerra et al. (prevalence of 12.4 %, $n = 346$), 16 years before the implementation of the national flour iron fortification program [27]. Nevertheless, these rates differ substantially from that estimated by the WHO in 2006 (29.1 %) [15]. Further, these anemia prevalence rates were also lower than those reported by studies in poorer municipalities from north, northeast, or mid-west regions of the country (22–50 %) [11, 14, 28, 29]. These contrasting findings agree with the evidence that social and economic inequalities are major determinants of regional differences in the occurrence of anemia [11, 30, 31].

We found no evidence of a significant fall in anemia prevalence between 2006 and 2008 nor when we compared it to those rates reported in pre-fortification periods [10, 27]. Therefore, despite an apparent trend of improvement in women's educational level, it is possible that causes other than iron deficiency may account for most cases of anemia in the studied setting. After assessing erythrocyte morphology patterns, we found that microcytic-hypochromic anemia, the most current blood picture of severe iron deficiency, accounted for a minority of cases in both studied years. However, we were not able to estimate the actual frequency of iron deficiency anemia among those women because of the lack of specific laboratory indicators of body iron status.

In fact, it is estimated that up to 40 % of patients with a normocytic-normochromic erythrocyte morphology pattern present actual iron deficiency [19]. Moreover, the coexistence of folate and/or vitamin B12 and iron deficiencies, which lead to the release of both microcytic and macrocytic erythrocytes in the peripheral blood, is associated with the normalization of estimated values of MCV [19]. Therefore, in this anemia of mixed causes, there is a marked anisocytosis and RDW determination may help the diagnosis [25, 32]. In our study, of all of the normocytic-normochromic anemia cases, almost half were associated with anisocytosis, hence suggesting that the women could indeed have been iron deficient. However, it is worth noting that if other nutritional deficiencies were also present in these cases, then dietary or pharmacologic interventions that use iron as the only measure could have a limited effect on anemia prevention and control [17, 33].

In addition, iron interventions could not have a strong positive impact on Hb concentrations in carriers of hereditary hemoglobinopathies. Nonetheless, from a worldwide perspective (and besides iron deficiency), the alpha- or beta-thalassemia traits should be considered as a common cause of low Hb, MCV, and HCM values among apparently healthy people [24]. Among Brazilians, thalassemia trait is a possible cause of a considerable proportion of mild microcytic-hypochromic anemia cases, most of which may be mistreated with iron supplementation in the clinical setting (if the nutritional iron deficiency is not a coincident complicating factor) [34–36].

It is difficult to differentially diagnose thalassemia traits from iron deficiency anemia without molecular or chromatographic tests, particularly when multiple conditions are present. However, the identification of anisocytosis may be useful because "pure" thalassemia traits usually lead to a production of erythrocytes with lower size variation than does the iron deficiency [25, 37–39]. In this sense, if we considered all of the diagnosed microcytic-hypochromic cases that were accompanied by normal values of RDW in our study, we could infer that one third of the women with this erythrocyte morphology pattern could not have severe iron deficiency at the time of the evaluation.

In Brazil, there are no data on the actual prevalence of hemoglobinopathies. However, many regional studies have shown that the alpha-thalassemia trait is not uncommon, especially among Afro-descendants or individuals who self-classify as black [40, 41]. Viana-Baracioli studied 696 pregnant women from 12 municipalities in the São Paulo State and found a prevalence of 10.7 % for hemoglobinopathies, with alpha and beta-thalassemia

traits being found in 6.75 and 1.29 % of all women, respectively [42]. Interestingly, alpha-thalassemia was apparently associated with ethnoracial classification, since it was found in 11.1 % of black women and 5.9 % of white women in Viana-Baracioli's study [42].

In our study, comparing women who self-reported as black with those who self-reported as white or mixed, we observed a higher frequency of microcytic-hypochromic anemia (univariate analysis) and lower mean values of Hb concentrations (multiple regression model). These trends have no great clinical significance because ethnoracial self-classification was not an independent predictor for the occurrence of anemia. However, data from the National Demographic and Health Survey (*Pesquisa Nacional de Demografia e Saúde* (*PNDS*)) show that non-pregnant women who self-reported as black have a higher prevalence of anemia on the national level [31]. International guidelines note that individuals with African ancestry indeed show lower Hb concentrations, irrespective of iron status, and should be evaluated with an ethnoracial-specific Hb cut-off to define anemia (1.0 g/dL lower) [17, 18]. However, whether this assumption is valid for individuals who self-report as black in our genetically admixed population is an issue that requires further confirmation.

Although the race, ethnicity, and skin color classifications that are frequently employed in epidemiological studies may correlate with ancestry, they are usually nonspecific and inaccurate measures of underlying genetic factors in highly admixed populations [43–45]. Moreover, because ethnoracial self-classification is a social construct, it may be a proxy for diverse environmental exposures [46]. Some studies have reported that Brazilians who self-report as black (followed by those who self-report as mixed) are more prone to have unfavorable socioeconomic conditions and to suffer discrimination [43], including in the care delivered by public health facilities [47]. Importantly, in our multiple regression model, black ethnoracial self-classification remained a significant predictor of lower Hb concentrations, even with the adjustment for current residence type, a potential indicator of socioeconomic conditions [48].

We also found that Hb was inversely correlated with gestational age at first prenatal attendance. Some researchers have emphasized the importance of commencing prenatal care at the very beginning of pregnancy (first trimester) in order to avoid pregnancy-associated risks [49, 50]. The women included in this study were presumably not taking iron supplementation. It is noteworthy that gestation progressively depletes body iron stores [1–3]; therefore, it is not surprising that the gestational age at the first prenatal attendance was an independent predictor of both Hb concentrations and prevalence of anemia. The fact that more than half of the studied women began prenatal care during the second or third trimester of pregnancy may have contributed to these findings.

A recent systematic review has examined the effectiveness of flour fortification programs in various countries [51]. According to this review, most of the published studies have been importantly limited by the decision to use anemia as the primary outcome, instead of evaluations of specific biomarkers of the nutrients (iron and folic acid) that were added to flours [51]. A positive aspect of our study is that, in addition to investigating Hb concentrations, we have used the parameter of erythrocyte morphology to characterize cases of anemia. Notably, these indices form part of the complete blood count (CBC) test, which is freely available at the Brazilian primary healthcare units [24]. On the other hand, the unavailability of serum ferritin measurements, which is the most specific biomarker of iron stores (in the absence of inflammation) [17–19, 25], precluded the assessment of iron deficiency prevalence, as previously mentioned. Other limitation is the fact that we used MCV, HCM, and RDW cut-off values that were not specific for pregnant women. Indeed, studies that apply erythrocyte morphology parameters to diagnose anemia in pregnant women are scarce [52–56].

The use of secondary data represents another limitation to our study because we were not able to confirm the quality of the information that was obtained from medical records. Moreover, data completeness could have influenced the representativeness of the selected samples since we excluded women with missing information regarding the inclusion criteria. Nonetheless, because women in the initial study sample and the final study sample did not differ in maternal age, gestational age, and hematological parameters, we believe that there were no large selection biases. The sample, however, was limited to apparently healthy women who attended the public health facilities in the region, and did not necessarily reflect the entire population.

Considering our data, we realized that we could not expect iron fortification to have a strong impact on gestational anemia in the studied population, since only a small proportion of women had severe iron deficiency as the sole cause of anemia (prevalence of microcytic-hypochromic anemia accompanied by anisocytosis). Nonetheless, in the logistic regression analysis, a non-significant 13 % lower odds ratio for anemia was estimated for women from the 2008 group, which could signalize a trend for a delayed effective impact of this program after 4 years from its implementation. However, our small sample size implied in a low statistical power to test difference in prevalences between the studied periods, arguing for larger studies and longer-term assessments.

In respect of the above-cited limitation, we were not also able to assess the effects of the year variable on the probability of having microcytic-hypochromic anemia or on Hb concentrations and the prevalence of anemia by gestational period. Nevertheless, it is worth noting that the frequency of anemia during the third trimester of pregnancy was almost two times lower in 2008 than in 2006, although the difference was not statistically significant ($P = 0.201$). Additionally, the proportion of all anemia cases that were microcytic-hypochromic was numerically smaller in 2008 (22.3 %) than in 2006 (40.2 %), although again the difference was not statistically significant.

Our results highlight the importance of performing detailed blood analysis in pregnant women attending prenatal care, especially among those with low Hb concentrations. Although the costs from these assays for iron deficiency diagnosis (e.g., serum iron, ferritin, and total iron binding capacity) may be prohibitive for large epidemiological studies, such tests could be prescribed during prenatal care by *Sistema Único de Saúde* (the healthcare system of the Brazilian government) [23]. The collection of this type of data would assist in the diagnosis of iron deficiency with and without anemia as well as cases of anemia caused by factors others than iron deficiency, in particular by nutritional deficiencies of folate, vitamin B12, or other micronutrients, and also by hemoglobinopathies.

Conclusions

This study has demonstrated a low prevalence of anemia among women enrolled in prenatal care provided by primary healthcare units of the Butantan Administrative Region (São Paulo municipality, Brazil) after 2 and 4 years that iron fortification program became mandatory. Among the diagnosed cases, the blood picture of severe iron deficiency (microcytosis, hypochromia, and anisocytosis) was not common. Therefore, it is possible that other nutritional deficiencies and hemoglobinopathies were frequent causes and/or complicating factors of anemia in this setting.

Abbreviations

CBC: Complete blood count; Hb: Hemoglobin; IBGE: Brazilian Institute of Geography and Statistics; MCV: Mean corpuscular volume; MHC: Mean hemoglobin concentration; PNDS: National Demographic and Health Survey; RDA: Recommended dietary allowance; RDW: Red cell distribution widths; WHO: World Health Organization

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Authors' contributions

EHSM contributed to the study concept, helped with the data collection and interpretation, and is the lead author of the manuscript. EDC helped with the statistical analysis, data interpretation, and manuscript review. SCS contributed to the study concept and helped with the data interpretation and manuscript review. JMP contributed to the study design and carried out the first statistical analysis. EF contributed to the study concept and helped with the manuscript review. CC contributed to the study concept and participated in the data interpretation, manuscript review, and obtaining the funding. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

The study protocol was submitted to and approved by the Ethical Research Committees of the Faculty of Pharmaceutical Sciences of the University of São Paulo (CEP protocol N 429) and by the Municipality Secretary of Health of São Paulo (SP), Brazil (CAAE 021.0.162.000-07).

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